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## UDP-Glucuronyltransferase

Report on the workshop conference  
of the German Society for Clinical Chemistry  
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in Schloß Auel

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**Summary:** A Workshop Conference on UDP-glucuronyltransferase was held in order to bring together different investigators specialising in a particular aspect of the enzyme. The intention was to find a basis for a closer cooperation between the different groups and to exchange ideas and methods making it possible to cross the hurdles facing those who work in the field of glucuronidation of endogenous and exogenous substances.

The topics which were chosen for the meeting covered the following aspects: methods of determination of UDP-glucuronyltransferase activity; problems relating to the origin of the enzyme and the nature of the substrate; application of assays of UDP-glucuronyltransferase in clinical chemistry; enzyme-substrate interactions and factors influencing the enzymic reaction; the rôle of UDP-glucuronyltransferase in the entero-hepatic circulation of glucuronides, regulation and induction of UDP-glucuronyltransferase, with a subsection on solubilisation and purification.

### UDP-Glucuronyltransferase

*Bericht über die Kleinkonferenz der Deutschen Gesellschaft für Klinische Chemie am 27. und 28. September 1977 in Schloß Auel*

**Zusammenfassung:** Eine Kleinkonferenz über UDP-Glucuronyltransferase wurde veranstaltet, um Wissenschaftler, die sich auf einen Teilaspekt des Enzyms spezialisiert haben, zu versammeln. Ziel war, eine Grundlage für eine engere Zusammenarbeit zwischen den verschiedenen Gruppen zu finden sowie Ideen und Methoden auszutauschen, um Hürden, die auf dem Gebiet der Glucuronidierung von endo- und exogenen Substanzen arbeitenden Wissenschaftlern entgegenstehen, zu überwinden.

Als Schwerpunkte des Treffens wurden folgende Aspekte ausgewählt: Methoden der Aktivitätsbestimmung von UDP-Glucuronyltransferase; Probleme bezüglich Herkunft des Enzyms und der Natur des Substrats; Verwendung der Bestimmung von UDP-Glucuronyltransferase in der klinischen Chemie; Enzym-Substrat-Wechselwirkungen und die enzymatische Reaktion beeinflussende Faktoren; die Rolle der UDP-Glucuronyltransferase im entero-hepatischen Kreislauf von Glucuroniden; Regulation und Induktion von UDP-Glucuronyltransferase; Solubilisierung und Reinigung.

*K. P. M. Heirweg* (Leuven, Belgium) discussed the need for a reliable assay of bilirubin glucuronyltransferase. The conjugation of bilirubin-IX in normal mammals *in vivo* shows considerable complexity: in addition to the attachment of bilirubin-IX (B) to one of several sugar moieties (xylose, glucose, glucuronic acid), mono- and di-conjugates can also be formed (homogenous and mixed conjugates). The presence of a variety of additional hexuronic acid-containing conjugates in body fluids of patients with cholestasis can be explained without invoking conjugating processes other than those observed for normal animals. Owing to the complexity of the conjugates and, in addition, to some rather bizarre chemical properties of bilirubin-IX (B) and its conjugates (in particular, the monoconjugates), progress in developing adequate assays for bilirubin glucuronyltransferase has been slow.

Specific difficulties are related to the following points:

1. The acceptor substrate, bilirubin-IX (B) and/or the product(s), may undergo changes during enzymic incubation (destructive processes and isomerisation by dipyrrole exchange).
2. So far, determination of product(s) has been based essentially on sampling procedures. Either transformation of conjugated tetrapyrroles into dipyrrolic azo-derivatives, or extraction of the tetrapyrroles followed by some separation method, have been used as the first step in quantitating the conjugated product(s). Both approaches present specific pitfalls, leading to artefactual changes which confuse the final interpretation of results.
3. Depending on the type of assay adopted, the final results may represent (a) an ill-defined fraction of conjugated B, (b) 'sugar transfer rates', or (c) 'bilirubin conjugation rates'.
4. Some assays allow one to estimate the ratio of mono- and di-conjugates formed, other assays do not.

A number of procedures can be adopted for presenting the acceptor substrate, B, to the enzyme, e.g. addition of a bilirubinate solution to a microsomal preparation, or previous solution of B in some organic solvent, or binding of B to some binding protein or other binding vehicle. Similar techniques have been employed in assays of glucuronyltransferase and other enzymes with various hydrophobic substrates. Major problems affecting the interpretation of the kinetic results are:

1. Which fraction of the substrate has to be used in the kinetic formulation of the enzymic reaction (factors such as protein-binding, effects on membranes of the added protein, distribution of substrate between incubation medium and membrane material, dimerisation or oligomerisation equilibria)?
2. Do any of the procedures for presenting acceptor substrate modify the properties of the enzymes assayed,

e. g. by changing the properties of the surrounding lipid matrix?

3. What can be the impact of such factors when more complex systems are studied (e. g. influence of inhibitors or activators)? A moderate excess of albumin in reaction mixtures protects bilirubin against the above mentioned transformations. Bilirubin, in the absence of albumin, precipitates onto the microsomal membranes, resulting probably in alteration of the properties of the membranes. Albumin prevents the changes by protecting the membranes from dissolution. The methods for the determination of bilirubin in serum, routinely employed in clinical laboratories, may be considered as reliable; the methods used to quantitate directly reacting bilirubin do not measure all the conjugates, e. g. conjugates of other sugars.

Clofibrate lowers serum bilirubin levels in patients with *Gilbert's* syndrome and enhances the bilirubin glucuronyltransferase activity as well as the Z-protein content in the rat liver. The investigations of *K. Kutz* (Bonn, F. R. Germany) revealed that treatment of rats with clofibrate, 100 mg/kg body weight daily intraperitoneally for two weeks, increased the activity of bilirubin glucuronyltransferase. This increase was similar to that observed by treatment of animals with 90 ng phenobarbital per kg body weight. It appears that clofibrate enhances the excretion rate of bilirubin into the bile. The bile of treated animals contained considerable amounts of bilirubin diconjugates. It was speculated that diconjugation takes place at the canalicular membrane. Whether the bilirubin lowering effect in patients with *Gilbert's* syndrome is due to the induction of bilirubin glucuronyltransferase remains to be explored.

*J. Fevery* (Leuven, Belgium) discussed how far the relative amounts of different bilirubin conjugates excreted in bile reflect the UDP-glucuronyltransferase activities measured in liver tissue. In a first approach, 11 animal species were examined. Only man, dog, cat and rat excreted diconjugated bilirubins and only liver tissue of the same four species formed diconjugates upon incubation *in vitro* with UDP-sugars. In contrast, the other animals excreted predominantly monoconjugates; incubation of liver tissue of these animals produced only monoconjugates. Furthermore, the ratio, bilirubin glucoside to glucuronide, present in bile also showed a correlation to the ratio of the transferase activities obtained in liver. A further correlation between the *in vitro* measured glucuronyltransferase activities and reported maximal rates of biliary excretion in different animals also suggest a close link between enzyme activities and biliary output. In a second study performed in man, it was found that the bilirubin UDP-glucuronyltransferase deficiency, as demonstrated in *Gilbert's* syndrome, *Crigler-Najjar's* disease and in neonatal hyperbilirubinemia, is expressed in bile by a decreased output of bilirubin diglucuronide. In the

most severe deficiencies (*Crigler-Najjar's* disease), increased amounts of unconjugated bilirubin-IX (a) were even present in the bile. It seems, therefore, that the *in vitro* measured transferase activities are a good reflection of the *in vivo* situation. However, in biopsy specimens from human cirrhotic liver widely scattered UDP-glucuronyltransferase activities, ranging from low to normal values, were found.

The solubility of various acceptor substrates in lipids is an important factor in the rate of formation of the glucuronic acid conjugate (*H. Paul, A. Illing*). Membrane perturbants, such as sonication, addition of detergent or alkane, or incubation with phospholipase or trypsin, result initially in increased UDP-glucuronyltransferase activity, frequently followed by deactivation. The partition coefficient between octanol and a buffer solution has frequently been used for investigating the solubility of chemicals in lipids. The relationship employed to compare the partition coefficient (or lipid solubility) of a compound with a biological response is the *Hansch* analysis which uses the equation

$$\begin{aligned} \log (\text{relative biological response}) \\ = \log (\text{partition coefficient}) + \text{constant.} \end{aligned}$$

This relationship has been found to hold for:

1. Unactivated enzyme activity (i. e. perturbation of membranes) in rat liver homogenates using carboxylic, thiolic and alcoholic acceptor substrates; aminophenols and aminobenzoates are included because they possess two conjugatable groups.
2. 'Activation factor' for treatment of rat liver homogenate with Triton X-100, using different carboxylic, thiolic and alcoholic acceptor substrates.
3. Enzyme activity in Triton X-100 activated rat liver microsomes for a series of substituted phenol acceptor substrates.

The activity of UDP-glucuronyltransferase *in vivo* presumably lies within the endoplasmic reticulum membrane. A lipid-impermeable barrier needs to be penetrated to allow access to the enzyme, which can be achieved by membrane perturbants.

For the selection of standard assay conditions for UDP-glucuronyltransferase, the ions required by the native, membrane bound and the partially purified enzyme must be present. Because of the accumulation of lipophilic aglycons in the membrane, the estimation of  $K_m$  values may be complicated.

A new, simple and sensitive method for evaluation of 3-hydroxybenz(a)pyrene glucuronidation was described by *A. M. Batt* (Nancy, France). It is based on the diminution of the amount of 3-hydroxybenz(a)pyrene after incubating it with UDP-glucuronic acid and rat liver microsomes. The fluorescence of 3-hydroxy(a)pyrene after addition of NaOH is measured for quantitation of the rate of glucuronidation. This method is less expensive

than a radiochemical method and suitable for routine use. The sensitivity of the assay was 0.1 nmol/mg protein per min; the  $K_m$  for the acceptor substrate was 0.09 mmol/l and 4.6 mmol/l for UDP-glucuronic acid. These values were obtained by untreated microsomes. Substitution of UDP-glucuronic acid by UDP-glucose does not lead to a conjugated product. Treatment of liver microsomes with cholic acid or Triton X-100 or digitonin inhibited glucuronidation of 3-hydroxybenz(a)-pyrene. Phospholipases A and C also were inhibitory. Trypsin, however, was almost without an effect. Administration of 3-methyl-cholanthrene, 20 mg/kg body weight on four consecutive days to rats (Wistar), enhanced hepatic UDP-glucuronyltransferase activity two to fivefold. Homozygous and heterozygous Gunn rats possessed 40 and 50% activity, when compared to the Wistar strain. Guinea pig liver microsomes had 2.5 fold higher activity than rat liver microsomes.

*B. Burchell* (Dundee, Scotland) was successful in purifying rat hepatic microsomal UDP-glucuronyltransferase to apparent homogeneity. Purification procedures included solubilisation with detergent (Lubrol 12 A9), precipitation with ammonium sulfate, DEAE, CM cellulose chromatography and DEAE-Sephadex column chromatography and finally the use of UDP-hexanolamine-agarose as affinity material. A 900-fold purification over the 10000 g supernatant was obtained, with a yield of 1.75%. The yield of pure protein can be increased significantly if the animals are pretreated with phenobarbital. No large differences in the properties of the enzyme between treated and non-treated animals were detectable. The pure enzyme formed 4.6 nmol 4-nitrophenol glucuronide/min · mg protein. Only one polypeptide band with a molecular weight of 57000 daltons was visible after SDS-gel electrophoresis. The pure enzyme generally behaved as an 'activated' form of the transferase. It was resistant to the action of phospholipase C, but this would still be consistent with phospholipid dependency if some phospholipids were tightly bound and not-accessible to the phospholipase. It was pointed out in the discussion that isolation of a catalytically active protein, demonstrably free of phospholipid-dependence, might be difficult. The protein could be stabilised not only by products of phospholipase action but also by traces of detergent used during solubilisation. An antibody has been prepared with which several questions of heterogeneity could be answered. The pure enzyme contained activities toward 2-aminophenol and 1-naphthol. The amino acid composition was not unusual.

*P. Czygan* (Heidelberg, Germany) reported that bile acid glucuronides are present in serum, bile and urine of patients with intra- and extrahepatic cholestasis. This finding was checked by measuring bile and UDP-glucuronyltransferase activity in the isolated perfused rat liver, in rat liver microsomes and in solubilised agarose-

bound enzyme preparations. Glucuronidation occurred with lithocholic, deoxycholic and chenodeoxycholic acid, but not with their taurine or glycine conjugates. The products are ester glucuronides, conjugated at the -COOH group of the aglycone, and not the C-3 position as originally supposed. Microsomal bile acid UDP-glucuronyltransferase was activated by Triton X-100. The enzyme could be induced by treatment of the rats with phenobarbital but not with 3-methylcholanthrene. Increased synthesis and excretion of bile acid glucuronides was observed after phenobarbital treatment of children with intrahepatic cholestasis. The glucuronic acid conjugates of bile acids are only found in urine during cholestasis, when high intracellular levels of the aglycons are present. Their formation and excretion may help to defend intracellular membranes against the lytic action of excess bile acids, thus serving as an important detoxifying mechanism.

*J. Magdalou* (Nancy, France) studied the influence of dietary proteins on rat liver microsomal UDP-glucuronyltransferase with *p*-nitrophenol as acceptor substrate. Wistar male rats were fed with a variety of protein diets: soya, different kinds of yeast, and casein, given in a proportion of 18% for continuous feeding. At the same time, other rats were fed a depleted (no protein) diet followed by repletion (18% protein). Among the different liver enzymes studied (transaminases, ornithyl-carbonyltransferase), UDP-glucuronyltransferase was noticed to be very sensitive to a change in protein source. With casein taken as the standard, soya protein was found to cause an increase in enzyme activity and a decrease in rat liver growth. The yeast (*Candida tropicalis*, *Kluyveormyces fragilis*), although ensuring normal growth, increased the activity of UDP-glucuronyltransferase. The effect of chronic feeding with these types of yeasts during 5, 14 and 28 days was investigated further. Yeast grown on methanol induced a transient increase of UDP-glucuronyltransferase and  $\gamma$ -glutamyltransferase activities. Feeding with yeast grown on paraffin led to low levels of UDP-glucuronyltransferase activity similar to that found with casein; repletion with lactic yeast induced a large enzyme increase in the specific activity of the enzyme. Lactic yeast which is very deficient in sulfur amino acids was not supplemented with methionine. On the basis of the result of the previous experiment, the role of sulfur amino acids on the regulation of UDP-glucuronyltransferase activity was then studied. The rats were fed a balanced diet, the only variation being in the methionine and cysteine content. Continuous feeding of the diet deficient in these amino acids induced a very large increase in the UDP-glucuronyltransferase activity (140%) of rat liver microsomes. Repletion feeding produced an even greater increase. With continuous feeding of the normal sulfur amino acid diet, UDP-glucuronyltransferase activity remained stable; yet on repletion with this diet, the values dropped to normal. It seems likely that the lack of methionine and

cysteine is related to the high UDP-glucuronyltransferase activity which may replace the sulfur-conjugation pathway. Some questions that arose during the discussion were: Does the latency of the enzyme in the membrane change? Are higher levels of the transferase enzyme produced? Why does gamma-glutamyltransferase increase? It was also suggested that the activity of the 'sulfur'-requiring conjugation system should be measured.

*M. L. Rao* (Bonn, F. R. Germany) reported results with the glucuronic acid conjugating enzyme, found in pig kidney microsomes. This enzyme conjugates oestrone selectively at C-3 position. The microsomal preparation of the pig kidney exhibited the highest specific activity of the glucuronyltransferase, while containing no  $\beta$ -glucuronidase, pyrophosphatase or hydroxylating activity under the incubation conditions used. The preparation was then chosen to study the reaction mechanism in detail. Increasing the concentration of either substrate (steroid or UDPGA) increased the affinity of the enzyme for the other substrate. The slope, *n*, from the *Hill* plot, was calculated to be unity for both oestrone and UDP-glucuronic acid. The  $K_{\text{oestrone}}$  and  $K_{\text{UDP-glucuronic acid}}$  were 6.6  $\mu\text{mol/l}$  and 254  $\mu\text{mol/l}$ , respectively. The oestrone glucuronyltransferase (UDP-glucuronate: 17 $\beta$ -oestradiol 3-glucuronosyltransferase, EC 2.4.1.59) of the pig kidney exhibits high substrate specificity; it is inhibited noncompetitively by oestradiol-17 $\beta$ , oestriol, testosterone, phenolphthalein and bilirubin, *p*-nitrophenol and *o*-aminophenol do not inhibit the glucuronidation of oestrone.  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$  were found to be nonessential activators. The effect of mercurials suggests the participation of thiol groups in the enzyme reaction. The enzyme does not exhibit a phase transition; the maximal activity was observed at 55 °C. One of the two products of the reaction, oestrone glucuronide, inhibits the enzyme competitively in the presence of increasing concentrations of UDP-glucuronic acid. The other product of the reaction, UDP, inhibits the enzyme noncompetitively with varying UDP-glucuronic acid concentrations. Under incubation conditions for the glucuronidation of oestrone, the enzyme catalyses the reverse reaction with oestrone glucuronide and UDP as reactants to the extent of about 0.4% of the forward reaction; this reverse reaction is also of a sequential type. In the discussion it was speculated that the pig kidney oestrone glucuronyltransferase may be a different enzyme protein.

According to *A. B. Graham* (Glasgow, Scotland), microsomal membrane bound *p*-nitrophenol UDP-glucuronyltransferase is modulated in two ways by the intact phospholipid bilayer membrane. Its full activity is dependent on an intact phospholipid environment, but it is restricted in activity in microsomal preparations showing latency by the impermeable nature of the membrane. Since a pure lipid-free glucuronyltransferase preparation was not available, an attempt was made to clarify the enzyme's interactions with membrane com-

ponents, the effect of temperature on the rate of glucuronidation and the thermotropic structural changes of membrane phospholipids. Latent enzyme preparations exhibit biphasic *Arrhenius* plots with a reversible discontinuity at 20–25 °C ( $T_c$ ), the activation energy of glucuronidation ( $E_a$ ) was higher at  $T > T_c$ . When latency is removed by perturbing the lipid phase of these membranes with phospholipase A, lysophosphatidylcholine, linoleic acid and Triton X-100, the *Arrhenius* discontinuity is greatly decreased or abolished and  $E_a$  at  $T > T_c$  is lowered. Glucuronyltransferase in guinea pig microsomal membranes prepared and washed with 0.154 mol/l KCl is not subject to latency and exhibits a linear *Arrhenius* plot from 5 to 40 °C with a high value of  $E_a$ . To obtain further support to these studies the polar headgroup area of the lipid bilayer of microsomal membranes and aqueous microsomal phospholipid dispersions was probed with the amphiphilic fluorophore 1-anilino-naphthalene-8-sulphonate; no phase change was detected between 5 and 35 °C. However, when N-phenyl-1-naphthylamine was incorporated into the hydrocarbon core of the bilayer, a transition centred at 21–25 °C was reported in native microsomal membranes, in membranes perturbed with phospholipase A and in dispersions of intact and phospholipase-treated microsomal phospholipids. From these results it was concluded that membrane permeability is rate-limiting in microsomal preparations with latent glucuronyltransferase. The *Arrhenius* discontinuity for glucuronidation in these preparations coincides with a structural change in the hydrocarbon region of the phospholipid bilayer; that latent glucuronyltransferase is associated with a deeply buried lipid domain.

Our knowledge concerning the formation of glucuronides of steroid hormones in liver diseases is rather confusing. To obtain detailed information about changes in oestrogen metabolism in cirrhotic livers, M. Höller (Bonn, F. R. Germany) studied the influence of cirrhosis on the uptake, metabolism and biliary secretion of oestrone and oestradiol-17 $\beta$  in the isolated perfused rat liver. Cirrhosis of the liver was induced by administration of carbon tetrachloride (i. p. for 10 weeks) and azathioprine (oral for 10 weeks). Histological examinations of the livers after chronic treatment with carbon tetrachloride and azathioprine showed characteristic cirrhotic pattern in all animals. Twelve weeks later the rats were used for perfusion of the liver. In some experiments the liver was post-perfused with an oestrogen-free medium. After perfusion the liver was homogenised; the homogenate, the perfusion medium and the bile were worked up separately and analysed for free and conjugated oestrogens. The functioning states of the livers were controlled by measuring oxygen consumption and oxygen partial pressure of the liver surface. ATP, ADP, lactate and pyruvate were determined in the liver; cytochrome P-450 was measured in the microsomal fraction. Hepatic clearance rates of oestrone and oestradiol-17 $\beta$ , the

production rates of different hydroxylated and conjugated metabolites and the biliary secretion of the two oestrogens in normal and cirrhotic livers were compared. The liver weights were not significantly altered under the experimental conditions. Compared to untreated animals, the uptake of oestrone by cirrhotic livers was diminished. The glucuronide formation of the two oestrogens, however, appeared to be normal when the diminished uptake was taken into account. Biliary secretion of oestrone glucuronide was markedly decreased, whereas a compensatory higher release of the glucuronide into the perfusate was observed. The decrease in the uptake of oestrogens could partly be explained by the formation of circulatory shunts in the cirrhotic liver. These observations are compatible with the hyper-oestrogenism observed in human cirrhosis.

G. Brunner (Göttingen, F. R. Germany) reminded the meeting that in severe liver disease phenolic compounds are not efficiently glucuronidated, and this results in their accumulation. These toxic compounds inhibit many endogenous enzyme systems and affect respiration; they also probably cause coma. To detoxify the poisons in the blood of such patients, attempts were made to replace their defective or deficient UDP-glucuronyltransferase. A partially purified UDP-glucuronyltransferase (acceptor *p*-nitrophenol and phenol) was solubilised by detergent treatment and purified 50-fold by DEAE-cellulose column chromatography, ammonium sulfate precipitation and Sephadex G-100 gel filtration. The solubilised enzyme was covalently bound to a variety of different carrier materials (e. g. acrylic acid-acrylamide). The carrier bound enzyme is very stable and can be used repeatedly for glucuronidation of phenolic compounds in aqueous solutions as well as in serum or blood. High concentrations of UDP-glucuronic acid were needed for glucuronidation of toxic substances. To obviate addition of substrate, UDP-glucuronic acid can also be entrapped in beads. Effective detoxification of rabbit blood was demonstrated with this material in an extracorporeal shunt.

K. W. Bock (Göttingen, F. R. Germany) stated that inducing agents differentially stimulate UDP-glucuronyltransferases in rat liver. For example, 3-methylcholanthrene mainly enhances the glucuronidation of 1-naphthol and 4-nitrophenol, whereas phenobarbital stimulates the glucuronidation of morphine, chloramphenicol and bilirubin. Rats were treated with 3-methylcholanthrene or phenobarbital; UDP-glucuronyltransferase, using 1-naphthol and morphine as substrates, was separated and partially purified. The presence of Brij 58 or Lubrol WX was essential. During the purification substantial amounts of phospholipids were removed. The molecular weight of the enzyme (1-naphthol as acceptor) was found to be about 58000 daltons. 3-Methylcholanthrene appeared to induce the transferase by de novo protein synthesis. Experiments with phospholipase suggested that detergents could replace phospholipids; the enzyme

is phospholipid dependent. Comparative studies on the formation of 1-naphthol glucuronide in the isolated perfused rat liver and in liver homogenates or microsomes indicated that the enzyme operates largely in a constrained form intracellularly. This constraint varies depending upon substrate and species. The constraint is probably due to a limited access of substrate and/or conformational changes of the enzyme which alter the effects of physiological modifiers, such as UDP-N-acetylglucosamine and UDP. The importance of the modifiers is demonstrated in the course of liver injury caused by  $\text{CCl}_4$ . During acute liver injury the constraint on UDP-glucuronyltransferase is released, while the level of the enzyme is decreasing. Release of the constraint leads to loss of the allosteric activation of the enzyme by UDP-N-acetylglucosamine, thus counteracting the activation due to membrane injury. Glucuronide formation in the perfused liver decreases but less than might be expected from the severity of liver damage. The content of UDP-glucuronic acid is not significantly altered under these conditions. In the discussion it was pointed out that the *Folch* extraction to remove phospholipids from membranes is inadequate, especially when the phospholipids are tightly bound.

G. J. Dutton (Dundee, Scotland) discussed the perinatal induction of UDP-glucuronyltransferase in chick embryo and in rat liver. In the chick embryo, transferase activity (acceptor *o*-aminophenol) developed 1–5 days after birth. Premature induction could be brought about by administration of pituitary extracts, corticotropin or glucocorticoids. Normal activation appears to occur as a result of secretion of endogeneous activator 2–3 days before birth. In the rat, the transferase activity with *o*-aminophenol, *p*-nitrophenol, 1-naphthol, serotonin or methylumbelliferone as acceptors, develops shortly before birth; this activity could be induced in cultures of foetal liver by adding dexamethasone to the medium. Transferase activity with a second group of substrates, e. g. bilirubin, oestrone, testosterone, morphine, chloramphenicol and phenolphthalein develops somewhat later and was not induced by dexamethasone. Thyroxine had no effect on *o*-aminophenol glucuronidation. The results were interpreted as follows: UDP-glucuronyltransferase activities toward a range of substrates in the rat which develop perinatally fall into two main 'clusters': those reaching maximal activity before or at birth and those reaching it after birth. Activities in the first cluster are induced by glucocorticoids and those in the second require different or additional factors.

Various metabolic disorders due to an imbalance of steroid hormone production or secretion are known in clinical endocrinology (G. S. Rao, F. R. Germany). The reasons for over-production of steroid hormones have been and are subjects of several investigations. The excretion of the hormones and the role of UDP-glucuronyltransferases under these conditions has not received the desired attention. Two questions were asked:

1. Are steroid glucuronyltransferases controlled by their natural substrate?
2. What are the effects of drugs and carcinogens on the activity of steroid glucuronyltransferases?

The experimental set-up consisted of studying the effect of ovariectomy and castration on the activity of the microsomal UDP-glucuronyltransferase, with oestrone and testosterone as substrates. The animals were then given oestradiol-17 $\beta$  and testosterone and activity was re-examined. Since it is known that drugs and carcinogens, e. g. phenobarbital and 3-methylcholanthrene, induce UDP-glucuronyltransferase which accepts non-endogenous compounds as substrates (*p*-nitrophenol, menthol, etc.), the effect of these two inducers on steroid glucuronyltransferases were also investigated. The results suggested that oestrone glucuronyltransferase is controlled by its substrate and that steroid glucuronyltransferases are not inducible by phenobarbital.

Discussing some recent results on reconstitution of drug hydroxylation and glucuronidation, O. Hänninen (Kuopio, Finland) pointed out that oxidation and glucuronidation reactions take place in sequence, resulting in the release of the glucuronide. In order to study the sequence, components of the microsomes were purified e. g. cytochrome P-450 and UDP-glucuronyltransferase. Starting with phenobarbital-treated rats, the liver microsomes were treated with deoxycholate, followed by ammonium sulfate fractionation. 7-Hydroxycoumarin was used as acceptor. Reconstitution experiments contained cytochrome P-450, the reductase, the transferase, phospholipid, UDP-glucuronyltransferase, phosphate- and Tris buffer, NADP,  $\text{MgCl}_2$ , 7-hydroxycoumarin, haemin and UDP-glucuronic acid. The deethylation of 7-hydroxycoumarin by the reconstituted system required phospholipid, but this appeared to be unsuitable for the transferase. Surfactants could be inhibitory to other enzyme systems and caution was recommended. When using such reagents it was stressed that alternative stimulating lipids should be looked for.

In conclusion, the results presented reflected aspects of the enzyme which could be of advantage to one or the other investigator. For the clinical chemist several presentations certainly were promising and revealed possibilities for applications in medicine. Intrinsic properties of the enzyme were highlighted by another series of lectures. The presentations on purification and induction of the enzyme showed that new avenues for the investigator are now open. The need for extensive communication between those working in this field was recognised by all participants.

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